# IDENTIFICATION OF PTEN MODIFIER GENES USING THE COLLABORATIVE CROSS MOUSE PANEL

An Undergraduate Research Scholars Thesis

by

RACHEL THOMAS

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# ABSTRACT

Identification of PTEN Modifier Genes Using the Collaborative Cross Mouse Panel

Rachel Thomas
Department of Genetics
Texas A&M University

Research Advisor: Dr. David Threadgill
Department of Genetics
Texas A&M University

Inactivation or mutation of phosphatase and tensin homolog (*PTEN*), a tumor suppressor gene, is implicated in unregulated cell proliferation, leading to tumor growth and the development of cancer. Identification of modifier genes, genes that alter the phenotype of another gene, of PTEN and their role in altering PTEN activity could provide insights into relationship between PTEN and cancer. The purpose of this study was to identify PTEN modifier genes by quantitative trait loci (QTL) mapping. To do so, we crossed transgenic mice that overexpress PTEN (super-*PTEN*) to lines of the Collaborative Cross (CC), a mouse population modeling human genetic diversity. We evaluated body weight at weaning as a surrogate for PTEN activity because it has been previously reported that super-*PTEN* expression is associated with reduced weight at weaning. Difference in body weight at weaning of super-*PTEN* pups compared to wild-type littermates was used for QTL analysis to identify modifier genes of PTEN.  This approach has identified candidate genomic intervals harboring PTEN modifier genes. Further studies will identify candidate genes and confirm these genes as modifiers. A pilot study has been initiated to verify that the effect of PTEN on body weight is indicative of the effect of PTEN on cancer susceptibility.

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# NOMENCLATURE

| | |
|---|---|
| *PTEN* | Phosphatase and tensin homolog (Protein) |
| *PTEN/Pten* | Phosphotase and tensin homolog (gene and transcripts for human/mouse) |
| CC | Collaborative Cross Panel |
| QTL | Quantitative Trait Locus Mapping |
| NCBI | National Center for Biotechnology Information |
| gQTL | Genome Quantitative Trait Locus Mapping |

# CHAPTER I

# INTRODUCTION

Cancer, a disease characterized by rapidly dividing, genetically abnormal cells, is responsible for a large number of deaths and poses a prevalent threat to human health ("Centers for Disease Control and Prevention," 2016). While the etiology underlying the development of cancer is not fully understood, it is known that inactivation of tumor suppressor genes is a crucial step in the development of tumors. Tumor suppressor genes are responsible for cell growth control; they code for negative regulatory proteins that regulate cell proliferation and prevent or slow the development of tumors. Therefore, when tumor suppressor genes are inactive, they are unable to regulate cell proliferation, contributing to the development of tumors (Cooper, 2000).

**Phosphatase and Tensin Homolog**

Phosphatase and tensin homolong (PTEN) is a tumor suppressor that works through negative regulation of the phosphoinositide 3-kinase (PI3K) pathway, which contributes to cellular proliferative signals (Figure 1). The *PTEN* tumor suppressor gene codes for a lipid phosphatase protein that dephosphorylates the 3 position of phosphatidylinositol-3,4,5-triphosphate (PIP3), a second messenger molecule which permits continued cell growth and proliferation, converting it back to PIP2 and preventing the continuation of the signaling cascade. Inactivation of *PTEN* through deletion or mutation is involved in the production of tumors in multiple types of cancers including prostrate, breast, melanoma and some brain cancers (Garcia-Cao et al., 2012).

*Previous PTEN Studies*

Previous studies have made progress in elucidating the role of *PTEN* in cancer. Studies evaluating *PTEN* in *Drosophila melanogaster* revealed it influences cell size and number. Studies of *PTEN* overexpression in mice showed that Tg(Pten)GPpp mice (hereafter called Super-*PTEN* mice) are smaller in size and show reduced tumor progression. Results of these studies showed that even slight changes in *Pten* expression could have a profound impact on the resulting cancer phenotype in mice treated with a carcinogen (Garcia-Cao et al., 2012). Distinguishing and understanding genetic modifiers, genes that can alter *PTEN* activity, could provide information on the causes, susceptibility and prevention of cancer.

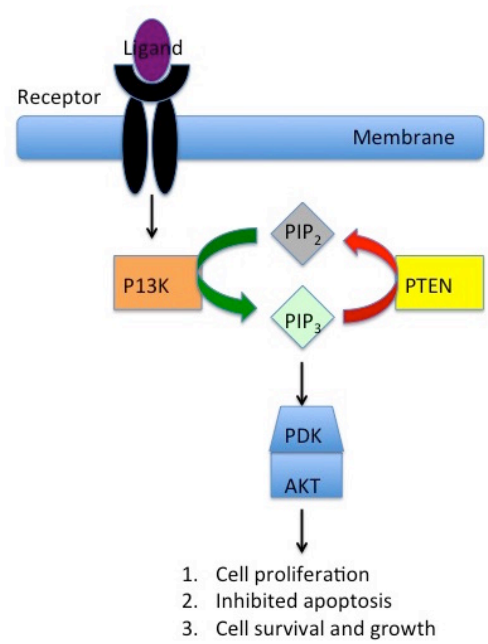


**Figure 1**. The PI3K-PTEN-AKT pathway. *PTEN* is a tumor suppressor gene that is involved in the P13K pathway. (Molinari, 2014)

## Modifier Genes

Modifier genes alter the phenotypic expression of another gene**.** They can affect the dominance, modification, expressivity and/or penetrance of phenotypes. Modifier genes account for differences between the genotype of genetic diseases and their phenotypic expression (Nadeau, 2001). Identifying *PTEN* modifier genes and studying their role could provide information on the relationship between *PTEN* and cancer.

## Studying Modifier Genes with the Collaborative Cross

The Collaborative Cross (CC), a panel of inbred mouse strains that contains levels of genetic variation similar to that of humans, is a new resource in mammalian genetics that can be utilized to identify modifier genes. The CC was derived from five inbred laboratory strains and three inbred wild-derived strains that were crossed and inbred for 20 generations to create mouse models that more accurately represent human genetic diversity (Aylor et al., 2011). The CC panel allows the identification of the role of novel genetic factors. Genetic modifiers can be identified by breeding CC mice with Super-*PTEN* mice that overexpress *PTEN,* and then using association mapping to identify genomic intervals that contribute to a selected phenotype, providing higher resolution mapping than other available resources. The CC panel allows for easier identification of potential modifier gene candidates through QTL mapping.

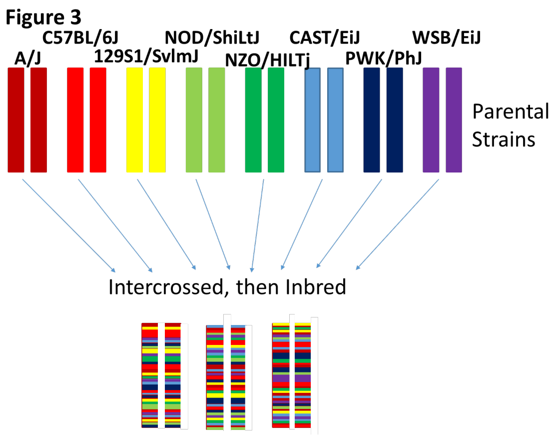


**Figure 2**: The CC was derived from five inbred laboratory strains and three wild strains that were crossed and inbred for a subsequent 20 generations

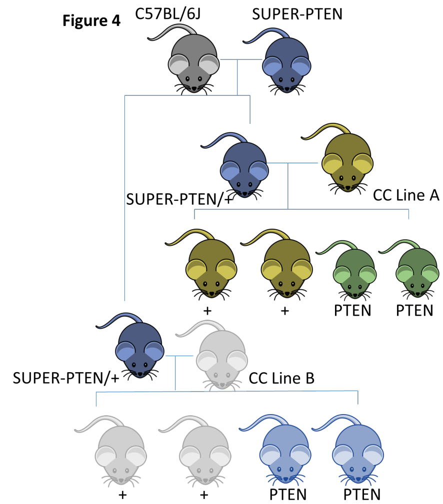


**Figure 3**: Breeding Scheme. Super-PTEN female mice were bred with male CC mice to produce offspring with various PTEN phenotypes.

## Objectives

We hypothesized that modifier genes can alter PTEN activity. Studying modification of PTEN function will provide information on the relationship between PTEN and cancer. The objectives of this project are to determine if *PTEN* modifiers exist using the CC population and observe the effects of the modifiers on cancer in mice.

# CHAPTER II

# METHODS

The purpose of this research project is to test whether PTEN modifier genes exist in mice and identify genomic regions harboring PTEN modifier genes.

**Identifying CC Strains with Modified PTEN Function**

The first part of the project involved breeding super-*PTEN* female mice with CC male mice of 33 CC strains (breeding scheme shown in **Figure 3**) and comparing the offspring with and without the *PTEN* transgene. Super-*PTEN* mice are distinguishable from their non-transgenic littermates by their reduced body weight at weaning, which is confirmed by genotyping. Average weight reduction of *PTEN* mice compared to their wild type littermates is 16%, but weight reduction can range from 0%-29% (Figure 4). This difference in weight was used to identify CC strains that possibly have modified *PTEN* function. Ear punch tissue samples were collected upon weaning and used for genotyping the mice.

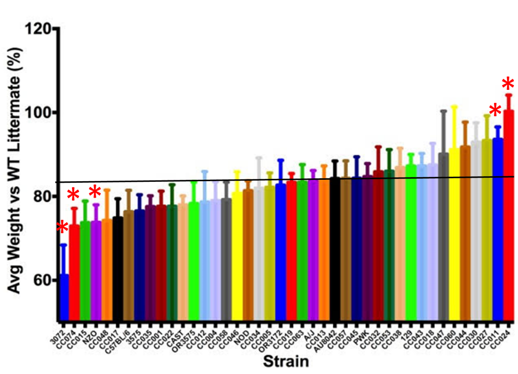


**Figure 4.** Weight at Weaning Data**.** The distribution of the weaning-weight phenotype is shown across 40 different CC mouse strains, including 10 strains that show a reasonable standard deviation from the average and 5 strains that deviate further.

## Quantitative Trait Locus (QTL) Mapping

The body weight at weaning for both super-*PTEN* and non-transgenic littermates was used as a surrogate phenotype for PTEN activity in quantitative trait locus (QTL) analyses to identify regions of the genome containing modifiers of PTEN activity. To control for differences across litters and strains, the weight at weaning phenotype was expressed as a proportion; the weight at weaning of male/female super-*PTEN* mice was compared to the weight at weaning of their wild type littermates.

QTL mapping was performed using gQTL. The QTL analysis produces LOD plots of statistical association (Figure 5). The higher peaks on the LOD plot indicate loci with more significant association to the PTEN-dependent weight phenotype. Candidate genes can then be explored in genome databases, such as the National Center for Biotechnology Information (NCBI), to identify genes present within the regions of significance with the highest LOD scores. The identified genes are possible candidate modifiers of PTEN; further tests will determine their effect on PTEN activity and function.

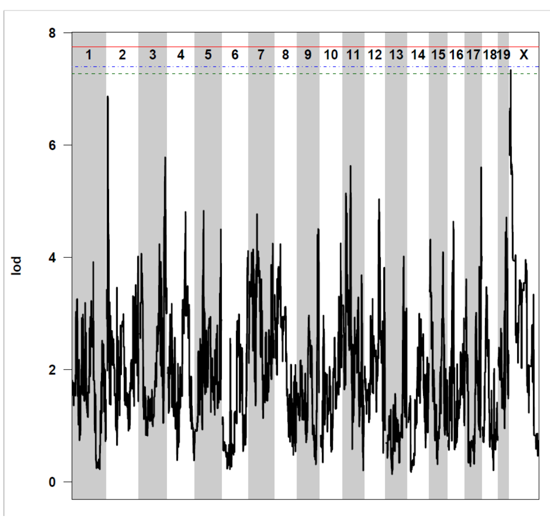


**Figure 5**. LOD Plot. Association mapping through gQTL produces LOD plots that are used to identify candidate loci.

# CHAPTER III

# RESULTS

QTL mapping identified genomic regions harboring possible candidate PTEN modifier genes.

**QTL Mapping Results**

The body weight at weaning from both super-*PTEN* and non-transgenic littermates was used as a surrogate phenotype for PTEN activity in quantitative trait locus (QTL) analyses and regions of the genome containing modifiers of PTEN activity were identified. To control for differences between the sexes, two separate gQTL analysis were performed using male and female data.

*Male Mice*

The gQTL analysis with data from the male mice identified a locus on chromosome 2, significant at a 90% level (P<.1) (Figure 6). This locus contains 8 genes, including Sfmbt2 located within the interval of the most significant LOD score, indicating that the *Sfmbt2* gene may have a significant association with the PTEN-dependent weight phenotype. Also present within the locus is Mir466, a gene encoding a microRNA known to regulate apoptosis (Druz et al., 2011), making this gene a promising candidate gene given the role of PTEN in apoptosis. This is potentially relevant to PTEN activity because inactivation or mutation of PTEN is implicated in inhibited apoptosis, continued cell proliferation and the development of tumors. Other candidate genes identified in the male mice include *Itih5*, *Itih2*, *Kin*, *ATp5c1*, *Taf3*, *Gata3* and *Celf2*.

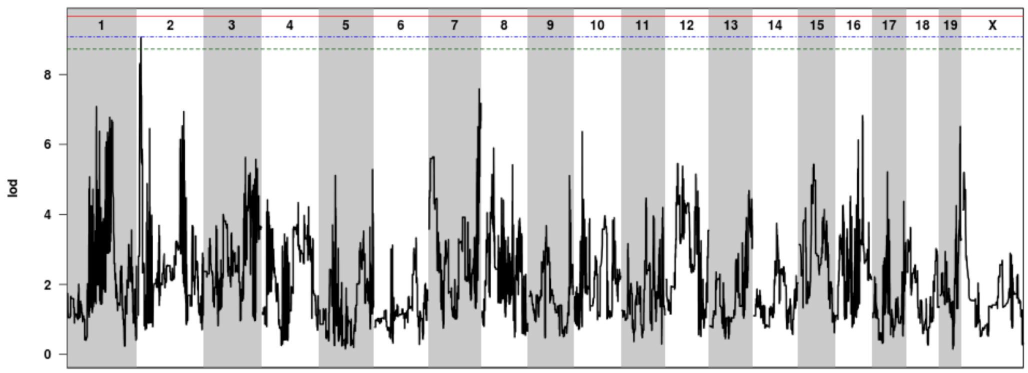


**Figure 6.** LOD Plot for Male Mice. Depicts the association between the surrogate phenotype (weight at weaning) and areas of the genome. The green and blue dashed lines indicate the significant thresholds at p=8.7 and p=9.0, respectively.

An allele effects figure generated through gQTL showed that the observed phenotypic effect at the identified locus is derived from three parental mouse strains, the CAST/EiJ, PWK/PhJ and WSB/EiJ (Figure 7). The colors on the graph indicate the genomic segments from different parental strains that are causing the association.

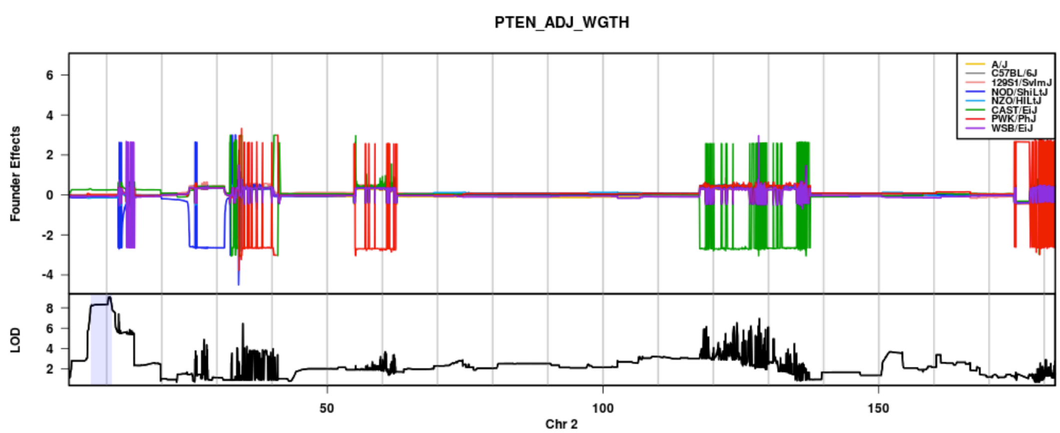


**Figure 7**. Allele Effects in Male Mice. Some mice strains contribute more than others to the observed phenotypic effect.

*Female Mice Results*

The gQTL analyses on female mice (both super-PTEN and their non-transgenic littermates) showed significant peaks on chromosomes 3(p <.1 ) and 12(p<.1) (figure 8). The locus on chromosome 3, the region within the peak (+/-1 LOD) contains 10 candidate genes, while the genomic region identified by a peak in chromosome 12 contains 8 candidate genes, including the transcription factor, *Gtf2a1*, which may be involved in regulating PTEN transcription (Huang et al., 2009).

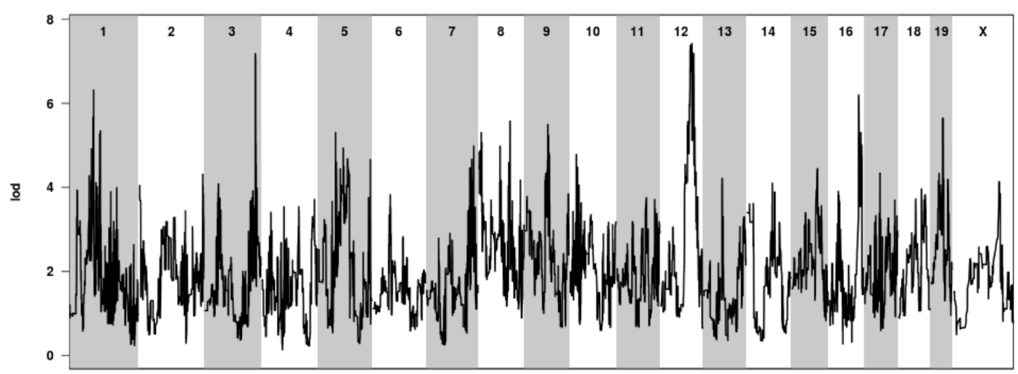


**Figure 8**. LOD Plot with Female Mice Data. QTL mapping produced a LOD plot showing areas of association on chromosome 3 and 12.

Additional weight at weaning data collection will improve the resolution of the LOD plots. With a higher resolution provided by the additional data, we hope to uncover additional significant loci associated with PTEN activity modification. Our current efforts are to confirm the identity of candidate genes from these loci as PTEN modifier genes and to validate their roles in the PTEN-dependent phenotype.

We have initiated a pilot project to confirm that the reduced body weight associated with PTEN overexpression is indicative of the effect of PTEN on cancer incidence and/or progression. To achieve this, we are utilizing three mouse cancer models: *Apc*$^{Min}$-induced gastrointestinal cancer, 3-methylcholanthrene-induced sarcoma, and PYVT-induced metastatic mammary cancer.

# CHAPTER IV

# CONCLUSION

We have identified genomic loci associated with body weight variation due to *PTEN* overexpression in male and female mice through QTL mapping. In male mice, nine genes including *Mir466* are located within the QTL on chromosome 2. In female mice, ten genes are located within the locus on chromosome 3 and eight genes including the transcriptional factor *Gtf2a1* within the locus on chromosome 12.

The next step of this project is to narrow down these gene lists to candidate *PTEN* modifier genes and to validate the extent of the modifier genes relationship with PTEN. Concurrently, three diverse mouse cancer models will be used to validate that this approach (using body weight as indicative of PTEN expression) plays a significant role in cancer initiation and/or progression. Understanding *PTEN* gene modifiers could provide insight into the causes, susceptibility and prevention of cancer.

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